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Review Article

Strategies for improving lipid production from various renewable residues using modern computational approaches – A review

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ABSTRACT
Over the last decade, there is increasing emphasis over "go-green" mantra for eco-friendly production especially over the fuel industry. The environmental hazard and vulnerability of fossil fuels due to prolonged usage has paved way for bio based fuel production. Lipids are the current trend of research significantly because of their capability to produce biodiesel. This review focuses on exploring the viable production process from various low cost substrates giving high yield. The researchers now target a wide range of microorganisms to synthesize lipids and thus biodiesel. However the volume of fuel generated by microbes is not sound enough to be marketed. This demands extensive research with various microbes and substrates. This work of literature discusses the merits and demerits of various microbial strains and alternative feedstock. This also throws light over the advances in process parameters, lipid extraction methods and conversion of lipids to biodiesel. The final sections of this review give an overview of the cost analysis and the future perspectives of biolipid production.

Keywords: Lipids, biodiesel, feedstock, residue, cost analysis

1. INTRODUCTION

The striking increase in the population across the globe, witnessed the increased need of water, food, energy and other primary commodities [1]. In the recent times, with the evolving awareness on environmental conservation, eco-friendly energy sources have replaced fossil fuels to meet the energy demand [2]. Lipids are produced in higher quantity by microorganisms. Lipids produced from oleaginous microbes are gathering attention as a potential substitute for conventional fuels like petroleum. Oleaginous microbes are species that accumulate lipids, specifically triacylglycerols (essential raw material for biodiesel production) to about 20-25% of their biomass weight. They can produce clean fuel and can serve as a renewable source of energy. Lipids can be manipulated through reactions to biodiesel [3]. Besides its advantages, the high production cost for production of biodiesel has inhibited the extensive application of this vehicle fuel [4]. This has inspired researches to work on innovative approaches to make cheaper production and affordable product in the market.

The production of lipid plies the use of many oleaginous microbes like microalgae, yeasts, bacteria and so on that act on various substrates to produce lipid and the produced lipid can be converted into biodiesel through trans-esterification [5]. Researchers are constantly working to bring out new, high yielding yet cost effective methods for fuel production from lipids. The low cost, easily available substrates actively experimented are papaya seed oil, karanja, cottonseed, tobacco, rubber seed, desert date, fish oil, neem, apricot seed, chicken fat, pork lard, beef tallow, waste paper, vegetable oil and waste cooking oil just to name a few [6]. There is ascending attention over the synthesis of lipids

from various substrates using oleaginous microbes, which can henceforth be transformed into biodiesel. Notably, microalgae can produce biomass and biodiesel with high lipid content (g/L) of 80% under specified conditions and methods [3]. Microorganisms like oleaginous microalgae- Chlorella protothecoides, Chlorella vulgaris, oleaginous yeast- Cryptococcus curvatus, Yarrowia lipolytica, oleaginous bacteria- Rhodococcus sp., Bacillus sp., Gordonia sp., Arthrobacter sp., and Acinetobacter sp., and many more have been trialed with the substrates and estimate the yield they produce [12].

Biodiesel is produced by transesterification of triglycerides [6]. This biofuel is said to also be produced from uncatalysed and catalyzed reactions among triglycerides. Catalyzed reactions could be homogeneous or heterogeneous which may therein involve acid, alkali or enzyme [7]. This is purely dependent over the type of catalysts involved [8]. Nano catalysis is an advanced technique where nano-particles are used for heterogeneous and homogeneous catalyst reactions as a catalyst [9]. In the case of carbonaceous catalysts, the waste biomass is transformed into a pyrolyzed char which upon activation act as a catalyst in the catalyst based reactions [10]. Supercritical transesterification and microwave-assisted transesterification is an alternate to catalytic transesterification [11]. Our review work focuses on bringing into light the different methods, substrates and microorganisms that are currently recognized in the production of lipid for their further conversion into biodiesel.

2. Biochemical pathway

The pathway and the mechanism involved in lipid accumulation of yeasts are deeply worked on extensively. The first stage in lipid accumulation is the nitrogen depletion. The increased activity of a nitrogen scavenging enzyme, adenosine monophosphate deaminase reduces the concentration of adenosine monophosphate. This reduction in concentration occurs right after the start of lipid accumulation. Consequently, activity of isocitrate dehydrogenase decreases. Isocitrate dehydrogenase is the catalyst in the conversion of iso-citrate to 2-oxyglutarate. Enzyme aconitase facilitates equilibration of isocitrate with citrate. This citrate is then moved into the cell outside the mitochondria. ACP citrate lyase that is present in the cell is notably the first organism that is responsible for the distinct metabolism portrayed between non-oleaginous and oleaginous yeast. In this pathway, it cleaves citrate to give acetyl- CoA and oxaloacetate. This oxaloacetate is furthered converted to malate. Pyruvate and NADPH is produced from malate under the influence of malic enzyme. This enzyme is essential to obtain high concentration high degree of NADPH concentration which is actively involved in production of fatty acid and accumulation of triacylglycerol. Taking into consideration that the required enzymes are available, acetyl CoA is progressed towards production of fatty acid and accumulation of triacylglycerol [13,14].

A metabolic shift occurs in oleaginous microorganisms during nitrogen depletion to support triacylglycerol accumulation. Excess of glucose with a high carbon-to-nitrogen ratio must be assimilated. Due to elevating citrate concentration, phosphofructokinase in the glycosis pathway is inhibited. Depleting nitrogen leads to increase of citrate. To further assimilate carbon, phosphofructokinase remains active forming a stable complex with NH_4. To maintain the carbon flow to give pyruvate, citrate regulates the pyruvate kinase.

3. Potential strains used for lipid production

Oleaginous microorganisms are known to be the common source of lipid production. Various organisms used as strains for lipid production is given in table 1. Upon metabolizing of various carbon substrates, internal accumulation of lipids occurs within the cells of these microbes. Oleaginous non-conventional yeast Yarrowia lipolytica, rich in hydrophobic substrates is considered as one among the most potential strains used for lipid production [15]. The capacity of storing large amount of lipids is possible because of the ability to break and use hydrophobic substrates. The growth and culture conditions of the yeast Y. lipolytica are greatly influenced by the protein and lipid composition in the lipid particles. There are proofs of enhanced particle size, lipid and protein composition conversion of glucose to oleic acid as a carbon source. Post sequencing and manipulation, harnessing the metabolic function for the purpose of bio-lipid production. Y. lipolytica displayed high potential as a lipid producing strain when used with carbon sources in various media like methanol, commercial glucose and molasses [16]. Strength and weakness of various oleaginous microorganisms are represented in Table 1.

Table 1: Strength and weakness of oleaginous microorganisms

Strain	Strength	Weakness
Lipomyces starkeyi	- appropriate candidate for single cell oil production - broad feedstock spectrum - produces glucanhydrolases - potential candidate for biomremediation	- difficult to genetically manipulate the strain
Cryptococcus curvatus	- fatty acid profile similar to conventional vegetable oil - highest intercellular lipid production with any modified yeast extract - broad feedstock spectrum- - high tolerance to lignocellulosic derived inhibitors - high lipid accumulation under low C/N ratio	- high production cost
Yarrowia lipolytica	- GRAS organism - High biotechnological potential - Can produce citric acid, organic acids and oil efficiently from glycerol - High biomass production with plant and animal fat	- exothermic fermentation process - Yield minimal when restricted to ambient conditions - Dry cell weight decreases at high temperatures
Trichosporon oleaginosus	- resistance to fermentation inhibitors - metabolises a large range of monosaccharides	- issues with sugar uptake - free fatty acid concentrations within the cell is low
Chlorella protothecoides	- High biomass and lipid production under heterotrophic conditions - 4X higher lipid accumulation via nitrogen limitation	- low the production efficiency - high production costs
Scenedesmus obliquus	- large cell size for more economical lipid production - rapid growth in waste water with high nutrient removal efficiency	- lack of research and details - high production cost

Rhodotorula glutinis is oleaginous yeast which is also stated to be a promising strain, with high growth rate and lipid accumulation when compared to moulds, algae and bacteria. In the process of fermentation, R. glutinis is demands huge quantity of water and nutrients for accumulating lipid and biomass. Remarkable growth of Rhodotorula glutinis is witnessed in high ratios of carbon and nitrogen [17]. Rhodotorula has a lipid reservoir and has been efficiently used for single cell protein production. It has been used for lipid production which is used in the treatment of MSG wastewater towards biodiesel production [18]. Whole volume composition of the fatty acids in the yeast biomass predominantly palmitic, oleic, linoleic acid makes it a source of lipid production. Besides this, they are used in food and cosmetic sectors due to their ability to produce carotenoids [19].

With the ability to convert economical fermentation substrates into bio-lipids, the oleaginous yeast Rhodosporidium toruloides is also a widely used strain for bio-lipid production. R. toruloides hold triacylglycerol up to 60% of its dry cell weight and additionally contains proteins as well [20]. These proteins are predominantly single cell proteins that are worth replacing the

conventional high cost sources of protein. So both microbial lipid and microbial protein production is possible from R. toruloides without discarding of the residues [21]. Simultaneous production of a possibly valuable by-product carotenoid adds additional merit to R. toruloides strain [20]. It works well along with a wide range of substrates, along with pigment production and high lipid content makes it a valid candidate for lipid biosynthesis [22].

Cryptococcus curvatus, oleaginous yeast is a popularly used strain for lipid production due to its reaction with a wide range of substrates. C. curvatus is a high energy density containing hydrocarbon precursors that yield lipid from cellulose [23]. It grows on disaccharides, hexoses and pentoses, leaving behind an exception of arabinose allowing lipid accumulation of 60% of dry cell weight [24]. It has minimal carbon to nitrogen molar ratio, greater tolerance towards inhibitors that are lignocellulose derived and a broad range of feedstock on which it can act [25]. Research works that demonstrate lipid production from cheap substrates on utilization of C. curvatus are constantly being worked upon [23]. The C/N ratio if above 20 affects significantly the lipid production from C. curvatus [26].

The microalgae Scenedesmus obliquus is a green chlorophyte which, due to its quicker growth and high volume production of lipid has been regarded as a deserving strain for lipid production. It has a larger size of cell supported with a 4-cells colonial structure that aids to economical harvesting. S. obliquus, with high nutrient removal capacity grows extensively faster in waste water [27].

The riboflavin is industrially synthesized using filamentous hemiascomycete Ashbya gossypii, it works on economical substrates and the downstream process is comparatively cheaper making it a desirable choice. Substrates like molasses and xyloses were utilized by A. gossypii for lipid production and the produced lipid was not more than 25% of the dry cell weight creating chances of impeding lipid synthesis from Ashbya gossypii [28]. Ease of growth of these filamentous fungi on industrial waste-based culture media makes it a desirable option. There are a wide range of sophisticated tools available for genetically manipulating A. gossypii [29].

Shorter span of growth cycle and larger content of lipid has made microalgal lipid Chlorella protothecoides an advantageous candidate over other strains. C. protothecoides can be manipulated using various sources of carbon to enhance the biomass and lipid production under heterotrophic conditions. After a span of 6 days of cultivation with corn hydrolysate in fermenters, C. protothecoides can be made to accumulate 55% of the dry cell weight with lipid. The facultative heterotrophic green alga C. protothecoides possess up to 4 times higher lipid content compared to the photoautotrophic C. protothecoides [30]. The variety of substrates it acts on, the ability to grow on limited media thereby reducing the degree of contamination considerably reduces the production costs and increases its suitability as a substrate. Also the cellular remains of C. protothecoides can function efficiently as a feed, hence its sales could account for economic convenience [31].

In the ocean, marine diatoms are the most abundant and bio diverse phytoplankton producing annually beyond 40% of oceanic organic carbon production globally. High biomass and lipid production rates have labeled diatoms as a relatively potential feedstock than other algal varieties. The quick growing Phaeodactylum tricornutum is a diatom, with large contents of fucoxanthin and lipid. Studies are constantly done to genetically manipulate the diatom Phaeodactylum tricornutum to give better yield of bio-lipids [32]. Another marine derived yeast obtained from the surface of a marine fish, Rhodotorula mucilaginosa TJY15a is capable of storing lipids obtained from the hydrolysate of cassava starch [33].

Lipomyces starkeyi, earlier reported as the nitrogen- fixing organism in soil is now found to hoard neutral lipids. A carbon rich and nitrogen lacking environment with an unbalanced mechanism is the biochemical significance behind the hoarding. Re-utilization of lipids was minimum in Lipomyces starkeyi compared to other oleaginous yeasts [34]. Holdsworth and Ratledge in 1988 proved that the storage volume of lipids is higher in L. starkeyi than R. toruloides, Candida curvata and Trichosporon cutaneum. Using extracellular carbohydrolases L. starkeyi breaks down carbohydrates while residing in soil and ensilage [35].

4. Selection of alternative feedstocks for lipid production

There is a constant debate over the high production cost of lipids. In order to overcome this, various measures are being taken, among which the selection of economically feasible and abundantly available feedstock is a vital one. These lipids are obtained from various classes of feedstock and the classification is described in detail in figure 1. The feedstocks that are largely

plied by current researchers for lipid production are mentioned in the table 2.

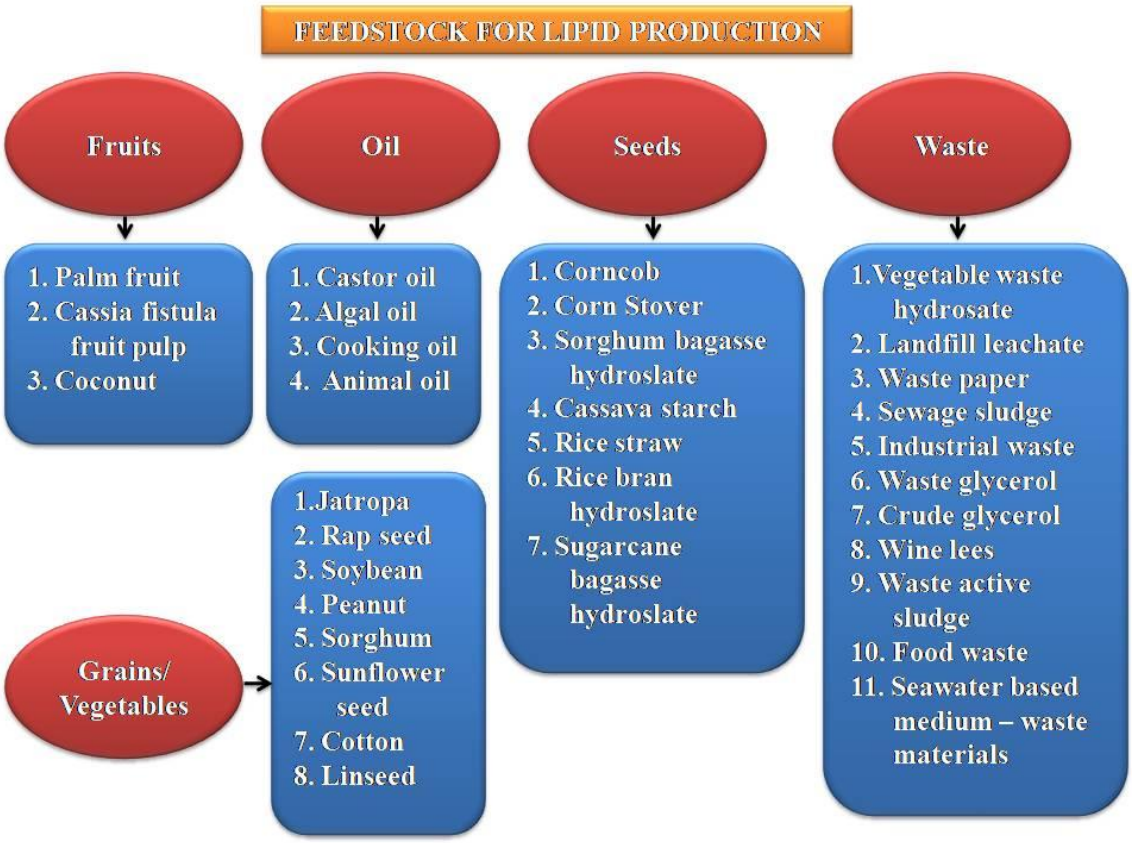

Fig.1: Potential Feedstock for lipid production

Table 2: Species, feedstock, process parameters for lipid production with lipid yield

S. No	Microorganism	Feedstock	Process type	Process Parameter	Reactor type	Lipid productivity (g/L/day)	Reference
	Oleaginous Yeast						
1.	Lipomyces starkeyi (Y)	sewage sludge	Batch	pH: 5 Temp: 30°C rpm:120	shaker flasks	6.4	[35]
2.	Cryptococcus curvatus	waste paper	Batch	pH: Temp: 30°C rpm:200	unbaffled conical flasks	200	[25]
3.	Yarrowia lipolytica	crude glycerol	Batch	pH: Temp: 28 °C rpm:240	Shake-flask	4.72	[73]
4.	Rhodosporidium toruloides yeast 32489	crude glycerol	Batch	pH: Temp: 30°C rpm:200	air-bath shaker flask	6.20 ± 0.27	[41]
5.	Cryptococcus sp.	corncob hydrolysate	Batch	pH: Temp: 25 °C rpm:	agar plates	7.6	[75]

6.	Trichosporon oleaginosus	crude glycerol	Batch	pH: Temp:28 °C rpm:170	shake flasks	0.19	[43]
7.	Yarrowia lipolytica	food waste-derived volatile fatty acids	Batch	pH: Temp:28 °C rpm:180	shake- flask	0.12 ± 0.02	[77]
8.	Yarrowia lipolytica	Waste Materials in Seawater-Based Medium	Batch	pH: Temp:28 °C rpm:200	shake-flasks	0.07	[79]
		Oleaginous Microalgae					
9.	Chlorella protothecoides (new)	crude glycerol	Batch	pH: Temp:28 °C rpm:220	shake-flasks	1.61	[76]
10.	Chlorella vulgaris FACHB-31	landfill leachate	Batch	pH: Temp: °C rpm:	shake-flasks	4.0498	[78]
11.	Chlorella protothecoides (old)	crude glycerol	Batch	pH: Temp:28 °C rpm:220	shake-flasks	2.07	[76]
12.	Chlorella protothecoides	biodiesel-derived crude glycerol	Batch Fed-batch	pH: Temp:28 °C rpm:200	shake-flasks	3.65	[30]
13.	Scenedesmus obliquus	glycerol	Batch	pH: Temp: 25 ± 1 °C rpm:120	filter-cap Erlenmeyer flasks	0.05966	[27]

4.1 Sugarcane bagasse

Currently the field of science has showed interest towards usage of cellulosic waste materials as a source of carbon. Due to the high quantity of annual produce, and the hydrolyzed sugars proving to be an efficient carbon source towards lipid production, lignocellulose has become a highly prioritized waste material. Taking into consideration the tropical countries, sugarcane bagasse is a highly preferred lignocellulosic material. The additional qualities that can put forth to validate its priority are the minimal lignin content and enhanced carbohydrate content, enormously availability at the site of sugar mill and the harvesting, transportation and storage being taken care of by production of sugar. Inorganic diluted acid forms of sulphuric acid and hydrochloric acid are plied largely for the sugarcane bagasse hydrolysis. These acids function as catalysts and break down the polymer chains by weakening the heterocyclic ether bonds formed by cellulose and hemicellulose between sugar monomers [16]. However acid hydrolysis can lead to degradation of sugars and formation of unnecessary by-products which demands additional purification and adds to the cost of production. Co-hydrolysis of lignocellulosic biomass which involves direct enzymatic hydrolysis post pretreatment can also synthesize lipids [36].

4.2 Food waste hydrolysate

The remaining leftover food and food preparative are termed as food waste and they are largely procured from residences and workplaces. The huge volumes of food waste generated are disposed leading to wastage of the surplus energy that the food waste contains and arouses secondary pollution. Recycling the organic components that are capable of being a potential nutrient source and an efficient raw material would definitely benefit the biotechnological industry. Recycling is brought about by the acid hydrolysis in order to recover the valuable nutrients present in the food waste. Bio-production of energy from food waste is a major focus of researchers today. Production of microbial lipids from such wastes is a commendable effort as the major constraint of

high cost fermentation substrate has been successfully overcome [21].

4.3 Waste activated sludge (WAS)

Waste activated sludge is a largely accumulated wastewater treatment process by-product which is generated in a quantity of 1200 kg of dry sludge for every 10,000 kg of treated sewage. Upon inhibition or reduction of methanogenesis, waste activated sludge is made to produce lipids by anaerobic fermentation. Using WAS hydrolysate as a carbon source, lipid accumulation in Chlorella protothecoides was studied [37]. WAS has a set of acid forming bacteria responsible for volatile fatty acid production. The changes in this microbe upon pretreatment in the anaerobic system contribute significantly to the volatile fatty acids (VFA) synthesis [38].With a high content of organic carbon and nutrients but less availability, WAS is a potential yet not widely explored feedstock for lipid production. Proper disintegration is needed as the nutrients are trapped in suspended solids and are insoluble. Solubilization can be achieved by alkali treatment, exposure to ultrasonic waves and hydrodynamic cavitation. Of these, alkali treatment is highly effective, yet the hydrodynamic cavitation is preferred largely due to its low cost, energy efficiency and easier scale-up [39].

4.4 Glycerol

A trivalent alcohol obtained by the degradation of the glyceride component of plant cell wall or the plant seed's reserve lipids is Glycerol. Synthesized on transesterification of animal fats and vegetable oils, glycerol has a wide range of applications in various industries. Implementing of microorganisms in the anaerobic fermentation of glycerol for the production of lipids and the conversion of the intermediate lipid into a value added bio-product is the current research trend [40].

4.5 Waste crude glycerol (WCG)

The by-product obtained on the production of biodiesel is the methanol rich crude glycerol. Mathematically, 10 kg of glycerol by-product is generated for every 100 kg of biodiesel production. Water, soap and catalyst are the other constituents present in crude glycerol besides methanol. Utilization of waste glycerol (WG) for biodiesel production is avoided in many industries due to the high degree of purification required. It is considered to have no profit. Also the disposal of the by-product crude glycerol is a difficult task making it an environmental threat. The raw materials used for biodiesel production are expensive. Hence the utilization of crude glycerol in the biodiesel production as a carbon source can prove to be a solution to all of these issues. Various advanced technologies are now established to make complete use of crude glycerol. The lipid production can be performed using the WG as a carbon source [27, 41, 42]. Off 113 yeast strains, 23 strains showed comparatively higher specific growth rate, biomass yield and production when treated with crude glycerol no matter the degree of the impurities present. These values are comparatively better than that obtained on treatment of microbes with glucose [43]. Upon cultivation of R. toruloides ATCC 10788 on waste crude glycerol, 21.16 g/L of biomass and 11.27 g/L of lipid concentration which was double and triple times in quantity respectively. The ability of the strain to grow on an impure media and still yield high amount of lipids showed the resistance and strength of the strain. The WCG obtained was found to be lipids rich in oleic acid and MUFA concentration which are essential for biodiesel production. By the use of such robust strains, it is possible to efficiently produce lipids from waste crude glycerol and divert them into biodiesel production [44].

4.6 Sewage Sludge

The disposal of sewage waste is a hectic task due to huge volume of toxic components it pertains. And the utilization of sludge is also not possible due to the above reason. But upon anaerobic conversion, the carbon contained in sewage sludge is converted to lipids. After finesse pretreatment, the sewage sludge could be implemented in production of lipids using different microbial strains like L. starkeyi, Chorella vulgaris etc. [35]. It contains certain essential elements for microbial growth like nitrogen, phosphorous and trace elements. With the addition of 25, 50, 100, 150 g/L of glycerol, a maximum lipid content of 9.35g/L, 10.13 g/L, 9.13 g/L and 9.03 g/L respectively. Combination of sewage sludge and glycerol proved to give better results than that which is obtained individually [45].

4.7 Waste paper

A potential feedstock of lignocellulosic biomass for lipid production is said to contain a carbohydrate content of 55-65%. Waste paper recycling is not 100% due to shortening of fibers resulting in depletion of paper quality. Hence such waste papers are exploited to produce various other products through multifarious

techniques [23]. With a composition of 5-15% hemicellulose, 40-80% cellulose along with lignin and proteins, waste paper obtained from cellulosic biomass is a major feedstock in lipid production. Though 400 ton of Waste papers are accumulated worldwide annually, its contribution to the production of lipids is minimally explored. Waste paper proves itself to be advantageous than the other feedstock in two aspects. Firstly, there isn't any need for pretreatment as the papers are already pretreated during pulping. Secondly, via enzymatic hydrolysis, it is possible to generate substrates like sugar-rich, nitrogen limited hydrolysates that can support lipid biosynthesis [25]. Various feedstock used for lipid production with advantages and disadvantages are represented in Table 3.

Table 3: Various feedstock used for lipid production

Feedstock	Advantages	Disadvantages
Sugarcane bagasse	• Low lignin and high carbohydrate content • Enormously available in tropical countries	• Acid hydrolysis is essential and this leads to formation of unnecessary by-products.
Food waste hydrolysate	• Contains a large variety of nutrient that supplement the production of lipids	• Acid hydrolysis is essential and this leads to formation of unnecessary by-products.
Waste activated sludge (WAS)	• High organic carbon and nutrients	• Less availability • Proper disintegration is essential
Glycerol	• Most important substrate for biodiesel production • High lipid yield	• Expensive • Requires methanol in large volumes for lipid separation
Waste crude glycerol (WCG)	• Efficient carbon source • Seminal molecule • Low cost carbon source	• Requires pretreatment • Alcohol, alkali and salt contaminations affect lipid production
Sewage Sludge	• High volume of metals	• Pathogens exist in SS may cause harm to environment and humans.
Waste paper	• No need for energy intense pretreatment to enhance the biomass recalcitrance. • Sugar-rich and nitrogen-limited hydrolysates can be generated via direct enzyme mediated hydrolysis • Extensively reduces feedstock related costs	• Paper type varies the product yield • Yield is slightly lower than enzymes • Deinking process is mandatory • Salt generated after pretreatment affect produce quality.

5. Fatty acid profile

Lipids are soluble in organic solvents and they are classified based on the polarity as polar and non-polar. Fatty acids are the constituents of both the lipid types and their subtypes and composition has an extensive impact on the quality and production of biodiesel. The fatty acids can be saturated or unsaturated characterized by no double bonds and single double bond respectively. Glycolipids, phospholipids are polar and are used for the formation of cell membranes by microalgae. Acyl glycerols and free fatty acids are non-polar lipids also called neutral lipids and are used as an energy source. Triacylglycerol having low unsaturation degree compared to other fractions of lipids is the main target to produce biodiesel. This is because they produce fatty acid methyl esters with high oxidation stability than the high unsaturated triacylglycerol. Hence microalgae

producing high volume of lipids but lacking TAG cannot be used for biodiesel synthesis [46].

The individual cell lipid composition is considered for biodiesel production and this varies from one microbial species to the other [46]. Biodiesel is composed of methyl esters obtained upon transesterification, after which they react with alcohol chains. These methyl esters could be methyl palmitate (C16:0), methyl stearate (C18:0), methyl oleate (C18:1), methyl linoleate (C18:2), and methyl linolenate (C18:3) which are exactly that of the profile produced by microalgae. The exact proportion for a good quality biodiesel synthesis from microalgae is 5:4:1 ratio of C16:1, C18:1, and C14:0, respectively. The properties of the biodiesel hence obtained are high cetane number, low iodine value, and low value of cold filter plugging point [47]. There is a decrease in the above mentioned properties with the increase in the number of double bonds. Chlorella sp., Scenedesmus sp., and Nannochloropsis sp., are the most studied and the most potential microalgal species for biofuel production (Sati et al., 2019).

Oleaginous yeasts have a lipid profile similar to vegetable oils. Species show a consistent lipid profile, provided it grows under uniform conditions. This profile, even for the same species, is subject to change with inducement of lipid profile, time and condition of cultivation. The yeast lipid profile has a domination of oleic acid which can be altered by selective inhibitors and the culture condition manipulation. Genetic manipulation of yeast can yield fatty acid profile suited for a specific application [48]. Table 4 represents the Fatty acid profile obtained from various oleaginous microbes.

Table 4: Fatty acid profile obtained from various oleaginous microbes

Microrganism	Palmitic acid C16:0 (%)	Stearic acid C18:0 (%)	Oleic acid C18:1 (%)	Linolenic acid C18:3 (%)	Linoleic acid C18:2 (%)
Lipomyces starkeyi	56	14	26	>1	-
Cryptococcus curvatus	19.51	10.86	50.13	7.28	0.93
Yarrowia lipolytica	25	52	56	6	26
Chlorella vulgaris	6	10.3	26.5	20.8	-
Chlorella protothecoides	19.03	2.35	48.21	1.54	1.29
Scenedesmus obliquus	27.39	11.88	32.08	8.11	9.08

Long chain saturated fatty acids of feedstock are chains of carbon atom chains saturated with hydrogen atoms which increase cetane number and reduce NOx emissions. Fatty acid chains interact with oxygen due to its unsaturation and double bond. The oxidative stability determines biodiesel shelf-life. Rhodotorula mucilaginosa grown in 5-L airlift bioreactor reported a fatty acid profile of C15:0 (3.4%), C16:0 (20.2%), C16:1 (1.2%), C18:0 (4.3%), C18:1 (42.6%), 18:2 (27%), and C18:3 (1.5%) when grown in the sea water. The lipid profile of Cryptococcus curvatus grown on synthetic media having 4 g/l acetic acid is C16:0 (15%), C18:0 (20%), C18:1 (40%), C18:2 (10%). Having a composition similar to vegetable oil, they are altered as the waste sludge is taken as a source for volatile fatty acid derivation for lipid production. The yeast R. kratochvilovae HIMPA1 grown on hemp seed extract generated a fatty acid profile of C16:0 (5.90%), C18:0 (25.10%), C18:1 (37.5%) along with C20:0 (22%), C22:0 (6.5%) and an unusual fatty acid C27:0, (3%). The same species when cultivated on nonedible lignocellulosic biomass of Cassia fistula L. fruit pulp, it gave a different fatty acid profile. Notably, when the same species was grown on paper pulp industrial effluent, in low temperature gave high quantity of long MUFA (45.43%) and PUFA (15.91%) chains that enhanced biodiesel quality with low CFPP along with good oxidative stability and cetane number [49].

6. Advances in process parameters

Altering degrees of unsaturation, variation in chain length are the major threats to manipulation of metabolic pathways. Engineered strain stability and the technique to achieve stable production are the secondary issues. The common notch for all these, are the enzymes which are largely membrane bound. The 3 noted interrelated genetic technologies comprise of cloning genes of essential enzymes, transgenic expression for high quality recombination of microbial oil and generating expressed protein by cloned gene modification. DNA recombination is brought about successfully in plants and recently progressing towards oleaginous

microbes. Fatty acid butyl esters (FABEs) in recombinant E. coli, wax esters in recombinant Pseudomonas citronellolis and FAME in recombinant S. cerevisiae are examples of recombinant fatty acid ester derivatives that are synthesized [50].

The information retrieved from the isolated enzymes and genes involved in lipid synthesis in the green alga Chlamydomonas reinhardtii are combined to chart out the manipulation strategies for various algal strains. The manipulation of metabolic pathways is done in various ways- obtaining a product using a rate limiting enzyme, over-expression, inhibition of competing pathways, vital enzymes being monitored by overexpressing transcription factor (tf) and site-directed mutagenesis. Through genetic manipulation, certain microbes promote enhanced lipid production while certain microbes do not show any impact. Taking this into consideration, strain specific metabolic engineering using modern tools is being studied [51].

Higher eukaryotes have their lipid synthesis pathway efficiently modified by metabolic engineering. The homology existing between higher plants and sequenced algal genomes makes it possible to apply the same lipid pathway modification is applicable for both microalgae and higher plants. When there is adequate substrate supply, there is a direct dependency between the flux of a metabolite and the preceding enzyme's activity. Over expression of this preceding enzyme, increases simultaneously both the enzyme activity and the flux of metabolite [51]. In the case of overexpression of ACCase of Arabidopsis thaliana, the TAG content of potato tubers amylopast increases five times. The acyltransferases are notable enzymes whose studies revealed them to be excellent targets for lipid pathway engineering. For example, in yeast gat1 mutant or two isoforms of LPAAT from Brassicanapus, the overexpression of glycerol-3-phosphate acyltransferase increased the volume of phosphoinositol and TAG [52]. This establishes the influence of a single enzyme over the entire biosynthetic cycle flux, validating these to be potential materials of metabolic engineering [51].

Instead of microalgal biomass harvesting, engineering microalgae to involve in direct secretion of lipids into the medium can waive off the excessive downstream processing time and costs. The development of cyanobacterium Synechocystis sp. PCC6803, a strain free of fatty acid is achieved by introducing a codon-optimized acyl carrier protein thioesterase and mutating it by deleting surface and peptidoglycan assembly protein. This led to weak phospholipid layers of cell wall causing increase fatty acid secretion and concentration [53].

Different culture conditions, the carbon to nitrogen ratio play an important role in variation of lipid composition. In the samples collected from Bacillus subtilis cultured in nutrient broth with C:N = 50 gave a difference in the fatty acid composition than the regular nutrient broth. Under increased C:N ratio, branched chain of fatty acid result a remarkable increase in the lipid production from 16.3% to 42.7% [50]. The fatty acid release from acyl-ACP is brought about by the introduction of acyl carrier protein and thioesterase enhancing lipid production. The fatty acid biosynthesis gene promotors by active TEF1 in the work by Runguphan et al., 2014 causing over-expression TAG enzymes, tail fatty acid producing enhanced TAG accumulation [54]. Advances in tools like metabolic pathway reconstruction tools, metabolic flux analysis, and computational modeling tools have scope for altering metabolic networks [55].

7. Extraction of lipid content from microorganisms

In comparison to higher order of plant and animals, microbial cells are found to contain higher volume of lipids in their cell wall [56]. The extraction of lipids from microorganisms is a very active area of research as review, as the determination of an efficient extraction technique is necessary for a maximum degree of lipid recovery. The various extraction techniques and their classification are mentioned in figure 2. Enzyme assisted, high pressure homogenization, bead mill, ultrasound assisted, microwave assisted, solvent extraction, Soxhlet method etc. are few of them [56]. In the following section I have stated the four extraction techniques that I found to be feasible, economical and high yielding.

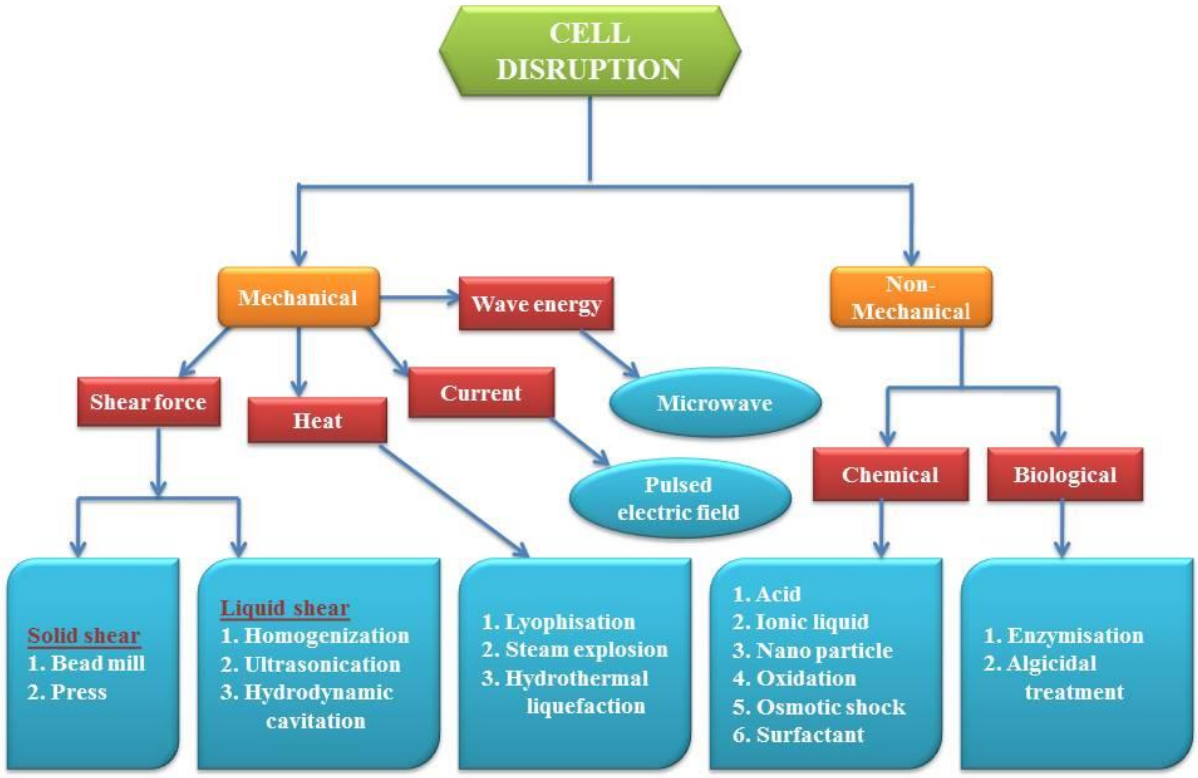

Fig.2: Extraction of lipid content from microorganisms

7.1 High pressure homogenization

One of the most widely applied physical cell disruption technique which is a shear based method for liquids. It involves low production costs and short residence times. Also it has low thermal degradation, heat transfer and pressure demand when compared to disruption using ultrasound. Efficiency of cell disruption varies with type of species and stage of growth on account of the varying cell wall rigidity [57]. In case of microalgae, high energy disruption technique is needed as the cells are small yet possess strong cell walls [58].

The high pressure generated is achieved by passing the cells through a small orifice of discharge valve using a positive displacement pump into a homogenizer. HPH can be applied to retrieve lipid from wet oleaginous microbes. The parameters to be considered are pressure, cell concentration and the number of passes that could lie between 60 – 150 MPa, 0.25 to 25% w/w CDW and 1 to 10 respectively. It is extensively used in agricultural and dairy industry to kill unwanted microbes and to breakdown the fat chunks respectively. Kumar et al. 2019 stated the extracted lipid content (%) to be 1.26 ± 0.06–40.06 ± 0.25 for Selenastrum minutum using HPH. There are several drawbacks in the application of HPH. Its suitability for high cell concentration where low energy is required remains its primary drawback. On using HPH for low cell concentration, there is a need to further drying to remove moisture. It is cumbersome to remove emulsion formed upon HPH in the processes following cell disruption. During scale-up for lipid production, high energy and high pressure necessities should considered [59].

7.2 Microwave assisted extraction

It is an indirect contact extraction technique based on the utilization of non-ionizing electromagnetic waves between the range of 300 MHz to 300 GHz. The waves interact with the polar molecules like water by traversing into the material and heating the total surface of material. Also a pressure gets generated within, resulting in cell disruption [60,61]. The rapid direction change of waves causes induction of electric and magnetic field. A localized heating is generated as friction arises when the polar molecules tend to align themselves along with the field [58]. This method is simpler when compared to other extraction methods. The

quicker heat and pressure generation results in a good volume of quality extract [62]. This is a preferred extraction technique when FAME is to be obtained from lipids owing to its simple and quick procedure [63].

An important factor involved in extraction using MW is the temperature. Extremely high temperature causes evaporation of solvent and depletion in the extraction. A condenser is used for maintaining the solvent volume to avoid solvent depletion. Under lower temperatures, disruption isn't efficient. Thick cell walled microbes need a long time of exposure with high temperature for disruption to occur [59] (Howlader et al., 2018).

Besides primary advantages like rapid extraction, higher yield, lower time and inexpensiveness, the other positive factors include lower need for organic solvent, no necessity for pre- drying, applicability for wet and dry biomass and swift energy transfer [60,61]. In case of wet biomass, research works have state that MW extraction increases the degree of lipid recovery. In Wahidin et al., 2014 involving the algae Nannochloropsis sp., lipid recovery using MW was 38.31g/100g CDW which was higher than 3.01 g/100g CDW obtained from the conventional water bath system [59].

In a continuous microwave assisted extraction of Scenedesmus obliquus, the mixture of water and microalgae was placed in a microwave for a certain time and temperature after which it was shifted to a water bath. After 30 min at 95°C, 77% lipid was extracted. Comparing biodiesel-ethanol (BD20 and BD40, having 20 and 40% biodiesel in ethanol) and chloroform ethanol with MAE at temperatures 80, 100 and 120°C, BD40 had higher lipid yield of the solvents at 100 and 120°C [58].

The development of free radicals and heat causes damage to the poly unsaturated fatty acids limiting the product quality and its utilization in large scale [61].

7.3 Pressurized gases for lipid recovery

This is a very efficient and promising technique yet hasn't been fully explored. Pressurizes gases like CO_2, N_2O, N_2 are used in the food industries for disrupting cell wall to restrict bacterial growth. This is found to be promising as it is cheap, easy to execute, nonflammable and non-hazardous. Different optimum conditions are set for different microbes. (Howlader et al 2018) [59]. Under exposure to pressurized carbon dioxide, oleaginous yeast Rhodotorula glutinis showed 40% increase in yield of lipid compared to raw untreated cells (Howlader et al., 2017) [64].

The process is initiated by dissolving CO_2 in the cell mass following which pressure and temperature are applied. Based on these two, some CO_2 dissolve in the suspension, traverse and enter the aqueous media via the gas- liquid interface. The CO_2 also works on the alteration of pH, both internal and external [65].

There are 2 states in which this extraction could be achieved- subcritical and supercritical pressurized condition. Of these, the former is preferred due to low energy used. The latter, though found to perform more efficient cell rupturing, is not widely chosen due to its expensiveness. In certain bacteria, this shift from subcritical to supercritical pressure doesn't have a bright impact on the cell disruption [66].

The factors that serve a vital role in disruption of a cell using pressurizes CO_2 are temperature, pressure, time of exposure, agitation, type of microorganisms etc. Increase of pressure, causes increased solubilisation of CO_2 which in turn increases the lipid recovery. Time of exposure is a factor completely microorganism dependent [59]. The pH also affects the lipid recovery indirectly by harnessing the activity of the cell. The metabolic enzymes are inactivated or lysed with the ascending or descending pH value leading to change in cellular activity [65].

7.4 Ultrasonication

Ultrasonic waves are mechanical acoustic waves with high energy. They break the strong attractive forces between molecules [59]. Ultrasound has been used for the cell disruption and lipid extraction of microalgae. Ultrasonication causes cell disruption by a 2 step process- 1) Cavitation and 2) Shock- wave progression [67]. An ultrasonic wave propagates in liquid medium producing alternate cycles of low and high pressure producing a huge cavity, which expands before collapsing causing high pressure and temperature. This process is called as Ultra-sonication. Ultrasonication attributes to formation, growth and collapse of gas bubbles. Ultrasonication consists of two phases namely rare fraction and compression phase. The bubbles grow during the rare fraction and are compressed during the compression phase, leading to the collapse of the bubbles [61]. A frequency of 20-100 kHz is generally used to produce high acoustic pressure or cavitations [57]. Ultrasonic cavitations are more intense at low frequency (18-40 kHz) than at high frequency (400-800 kHz). These extreme pressure and temperature causes the

components of the algae to break. Among different methods of cell disruption methods, ultrasound technique has proved the best [62]. Though the ultrasonic waves are extensively used in the food processing industry, it is also used for lipid recovery. This method is also used to disrupt the cell walls followed by lipid extraction using a solvent or it can be used in trans-esterification process to produce biodiesels [59]. Kumar et al. 2019 stated the lipid content (%) to be 49.82, 46.81, 11.73 for the Chlorella vulgaris, Scenedesmus dimorphus and Nannochloropsis sp. respectively by ultrasound assisted extraction.

Ultra-sonication can cause both physical and chemical changes through cavitation process. Physical cavitation results in micro-turbulence and shockwaves with amplitudes of 20-50 bar. Micro turbulence leads to efficient mixing of biomass and solvent leading to cell wall rupture. This in turn improves lipid yield during lipid extraction. Moreover intense turbulence produced due to ultrasound pushes away extracted lipids from the surface of the microbial cells. And a constant concentration is maintained for continuous diffusion of lipids [63]. This method improves material transfer rate supporting the extraction of the lipids. Ultrasonic assisted extraction technique with sound waves of frequencies higher than 20 kHz is used. This high intensity technique is used for the better extraction of lipids from the microalgae. Ultrasonication has been widely used for protein synthesis, chemical synthesis, disinfection and cell disruption with reduced chemical additions [60].

Besides these, there are several chemical and enzymatic methods, which are used for extraction of lipids. Though they are efficient in lipid recovery, there are certain setbacks that hinder severely in their usage in scaled up lipid recovery. The cost of enzymes is quite high making it inappropriate for large scale usage. There is additional purification steps necessary to treat the lipid that are chemically extracted resulting to added production costs. Also certain cells get lysed when exposed to chemicals. These statements justify their inappropriateness for usage [59].

8. Conversion of microbial lipid into biodiesel

Various works are conducted on the oleaginous microbes and the production of lipids. A majority of these works are directed towards the utilisation of these microbial lipids into production biodiesel. The high percentage of oleic acid contributing a suitable fatty acid profile, oleaginous yeasts are the most picked microorganism for biodiesel production. Added attractiveness is the ability of it to overcome the shortcomings of first-generation biofuels [48].

Biodiesel is a non- hazardous, eco-friendly biodegradable liquid fuel. It has a fatty acid long chain lined up by mono-alkyl esters. The production of biodiesel from lipid is achieved through transesterification of lipids [9]. Transesterification is the derivation of oil from plant, animal or oil-producing microbes for reacting with alcohol, preferably methanol to produce fatty acid methyl esters (FAMEs) which is nothing but biodiesel. This can be executed in either at high temperature and high pressure environment or in a mild environment in the presence of a catalyst. This catalyst could be homogeneous, heterogeneous, acid, base or enzyme [68].

Acids like Hydrochloric acid and sulphuric acid in the laboratory level, function as catalysts. The reason for not being able to use them in large scale is the constant attention that needs to be provided due to their violent nature and their excessive consumption of methanol. Bases like sodium hydroxide and potassium hydroxide as industrial level catalysts. Enzymes like lipase have showed better activity than acid or base, but are very expensive. Cost management of lipase is encountered by designing a cheaper production process, enhancing lipid efficiency and increasing lipase reusability. Reusing lipase is done by immobilisation in various carrier materials which can still retain lipase activity and stability [68].

Replacing the conventional biodiesel production techniques, nanocatalysis have now become a research trend as they combine the advantages of homogenous and heterogeneous catalyses thereby reducing their drawbacks. The use of nanoparticles as a catalyst to fasten a chemical reaction, which here is the transesterification step is the principle of nanocatalysis in biodiesel production. Gupta et al., 1724, showed 93-96% biodiesel yield by CaO nanocatalyst. Combined catalysis have shown better results as in Tahvildari et al., 2015 where CaO coupled with MgO gave 98.95% biodiesel yield which is higher than a single catalyst. CaO/Fe_3O_4, a solid base magnetic nanocatalyst showed good results in production of biodiesel date palm seed oil. In case of biodiesel extraction from olive oil, ZnO nano-rods have better efficiency than conventional ZnO. Catalysts of Potassium bitartate with zircona as the support material are also developed. There are many researches and

review works presented in the perspective of nanocatalyst mediated biodiesel production. The size and geometry of the catalyst, the chemical and physical environment, surface functionality and composition are factors that have an impact on the activity of nanocatalyst [9].

Besides extraction, ultrasonication is a technique also involved in the transesterification of lipid to biodiesel. The indispensible methanol in most cases stays behind as a by-product. The cavitation phenomenon induced by sinusoidal mechanistic waves. The bubbles hence generated function as a micro-reactor for methanol and the lipid transesterification. When these bubbles break open with a high temperature and pressure being generated within the micro-reactors in the ranges of 1000 – 10000 K and 10 – 500 MPa respectively, vaporization of methanol and lipid occur following which an effective mass transfer takes place enhancing the reaction rate and efficiency. Through ultrasonication, the methanol to lipid molar ratio is brought down to 6:1 from 12:1. The reaction time is reduced to 30 from 60 min and the temperature decreased to 35°C from 50-60°C [69].

9. Future Prospects

The biosynthesis of lipid is a stream of active research where there is scope of advancement in every perspective. Amendments could be done on the raw materials, processes, product, and microbial strains with the notion of a novel, cost efferent, high quality, and environmental friendly advancements.

Molecular engineering tools are developed via synthetic biology, genome editing and systems biology. There is a future work space for convergence of these developing molecular engineering tools with the genes involved in lipid production as they are to be manipulated to improve the microbial strains. This can lead to efficient and economical strains being produced for lipid production. There is also scope for synthesis of quality essential by-products along with bio-lipid production and biodiesel synthesis to make it economically beneficial [70].

The future works should also be focused on sewage sludge as the raw material. Though it is a low cost substrate, there are certain disadvantages that restrict its use. The pretreatment which comprises of collection, dewatering and drying is very expensive. As the lipid extraction involves usage of organic solvents, the production cost is hence increased. Solvent sludge ratio, type of sludge and solvent has impact on the efficiency of lipid production and the cost. So research on these factors is needed to establish an economical and efficient lipid extraction from sewage substrate. Finally a perfect method for biodiesel production from sewage sludge needs to be found and made to possibly function similarly with other substrates [71].

Besides looking into production cost and energy, another important aspect to be looked into is making the raw materials, processes and product eco-friendly. On this note, green solvents have replaced many organic solvents in case of extraction. Yet the technicality and application in lipid recovery has to be researched. Optimization of operating parameters, synergistic effects of different combination of methods, green solvents and new large scale working strategies, wet biomass extraction and direct transesterification are the vast range of future prospects [46].

10. Cost analysis of microbial lipid production

The production of biodiesel is expensive due to various associated parameters. Cheirsilp et al., 2013 stated that the major reason for the elevated production cost is because of the raw materials which account upto 70-75% of the total cost [72]. In case of microorganism based biodiesel production from lipids, the usage of costly substrates and nutrients for microbial growth, large volumes of methanol for transesterification and extraction of lipid hype the cost of lipid production. Alternative feedstocks that are less expensive like industrial wastes and by-products and those that are easily available are chosen to overcome this set back. Also, through the advances in genetic engineering, microorganisms are manipulated to reduce the energy consumption during growth, enhance the ease of harvesting and increase the lipid yield. Another approach is to reduce the methanol demand but maintain transesterification rate [69]. Hence for cost control, firstly it is very much essential to work on looking out for low cost substrates that could yield significant volume of lipids yet require minimal or low pretreatment and purification. Secondly, microbes should be engineered in such a way that they could consume minimal energy and nutrients but still give high lipid yield. Finally, bringing a process parameter that could work on minimum energy giving greater results could additionally contribute to cost cutting. Combining all of the three factors will generate an overall cost efficient process for lipid production.

11. CONCLUSION

We have comprehensively overviewed lipids and the various important aspects of lipid synthesis and the further transesterification to biodiesel in our work. This review concentrates over the various low cost alternative feedstocks for lipid production and on microorganisms that can yield significant quantities of lipid. Also the most suited lipid extraction processes and their transesterification to biodiesel is reviewed. Consequently, the recent advances in the processes to ease the production are also discussed. To conclude, though various cheap substrates, microorganisms and efficiency of process are being trialed, the goal is yet to be achieved. Formulation of an overall cost-effective production process for lipid and hence biodiesel, will increase the biodiesel demand in the fuel market in the near future.

Declaration of conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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